Comm.
Dr. Loosli
Dr. Sommers
Dr. Wyatt

CHRONIC PULMONARY DISEASES

#76 2C

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N.Y. 10022
(212) 421-8885

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MAY 3 1973

Application for Research Grant
(Use extra pages as needed)

Date: April 12, 1973

1. Principal Investigator (give title and degrees):
Albert H. Niden, M.D., Professor of Medicine, Director Pulmonary Disease Section

2. Institution & address: Drew Postgraduate Medical School
1620 East 119th Street
Los Angeles, California 90059

3. Department(s) where research will be done or collaboration provided:

Department of Medicine, Pulmonary Disease Section.

4. Short title of study:
Effects of Cigarette Smoke, Noxious Fumes and Drugs on the Terminal Airways

5. Proposed starting date: July 1, 1973

6. Estimated time to complete: Through June 30, 1976

7. Brief description of specific research aims:

a) To elucidate the role of the large alveolar cell, non-ciliated bronchiolar cell and monocyte-macrophage system in pulmonary phospholipid metabolism. Phospholipid metabolism will be studied by utilizing autoradiographic techniques to localize and follow the subcellular formation of phospholipids from fatty acid precursors. The effect of various stimuli e.g. cigarette smoke, noxious fumes and drugs on phospholipid metabolism by these cells will be observed.

b) To study pulmonary capillary permeability and the factors affecting it. By using E.M. tracer protein molecules of known sizes (horseradish peroxidase at 40 A°, hemoglobin at 65 A°, Dextran 40 A° to 200 A°) one is able to investigate the effects of intravascular pressure and damage to the capillary endothelium on pulmonary capillary permeability. The effects of air pollutants, drugs and cigarette smoke on pulmonary capillary permeability will thus be studied.

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8. Brief statement of working hypothesis:

a) Three cell types in the terminal airways have now been shown to actively synthesize phospholipids--the large alveolar cell, the non-ciliated bronchiolar cell (Clara cell) and the monocyte-macrophage. Only from indirect evidence, the large alveolar cell is generally assumed to be the source of pulmonary surfactant. The role of the other cells--in particular--the Clara Cell in this process is not known. Finally, the effect of a marked increase in the monocyte-macrophages in the lung on the measured alterations in phospholipid metabolism (presumably surfactant) in pathologic states has not been considered by previous investigators. It is proposed that the Clara cell is intimately involved in surfactant production and marked changes in cell populations as readily observed in many pathologic conditions could effect phospholipid content and metabolism in the lung independent of alterations in pulmonary surfactant. The latter would lead to false conclusions by others as to presumed alterations in surfactant metabolism.

b) The capillary endothelium appears to be sensitive to various noxious stimuli leading to an increase in pulmonary capillary permeability. Thus the common pathway for the various disorders leading to the Adult-Infant Respiratory Distress Syndrome ("Stiff Lung") is alveolar capillary endothelium damage with a transudation of fluid into the interstitium and alveoli.

9. Details of experimental design and procedures (append extra pages as necessary)

a) Pulmonary Phospholipid Metabolism - The ultrastructural responses of the Clara cell, large alveolar cell, and monocyte-macrophages to various stimuli will be studied. Mice and hamsters will be exposed chronically to cigarette smoke (when Walton Horizontal Smoking machine becomes available - see Progress Report) or placed in a plastic, environmental chamber and exposed to 15% oxygen, 100% oxygen, 5% CO_2, nitrogen dioxide, etc. Tritiated fatty acids will be complexed with serum albumin and injected. Since 40 to 60% phospholipids are extracted by normal fixation -- embedding for E.M., present techniques will be improved to reduce the loss (see progress report). In addition, E.M. frozen tissue technique will be developed for E.M. lipid autoradiography. Standard techniques for light and E.M. autoradiography will be used to follow the synthesis of phospholipid in the lung at 2, 5, 10, 20, 60, 240 minutes after injection. (We have shown active incorporation occurs in the first few minutes following injection.) Using standard TLC techniques the tag will be localized among the lung lipids. Because of the rapid turnover of lung lipids, it is questionnable that a pulse labelling occurs in vivo so that it might be difficult to sequentially follow phospholipid metabolism in the lung with such technique. Therefore, the in vivo studies will be compared with in vitro pulse labelling of lung tissue slices (see progress report). The autoradiographic-biochemical studies will be correlated with direct measurements of surfactant activity extract from experimental lungs as measured on surfactometer (Kimray-Greenfield).

b) Pulmonary Capillary Permeability - Horseradish peroxidase (HRP Worthington) with a diameter of 40 A°, 0.5 mg in 0.05 ml saline, will be injected intravenously into tail vein of control and experimental mice. The mice will be sacrificed four minutes after injection. Tissue will be prepared for ultrastructural visualization of HRP by the Graham-Karnovsky method as well as for routine light and electron microscopy. Effects of exposure to cigarette smoke, 100% oxygen, 5% CO_2, as well as the injection of drugs (epinephrine, alloxan, ANTU, serotonin) on pulmonary capillary permeability will be studied. In later studies, it will be necessary to use larger animals in order to monitor pulmonary artery pressure to assess the relative role of direct effects on membrane permeability versus secondary effects from increased intravascular pressure. In all experiments, lung weight/body weight ratios will be used to assess degree of vascular congestion and/or edema. (This is insensitive to early changes in pulmonary capillary permeability (see Progress Report). In particular, we are interested in examining the reversibility and prevention of oxygen toxicity. Changes in pulmonary capillary permeability are an early sign of oxygen damage and appears to be a sensitive model for studying this proglem (see Progress Report). Hemoglobin (65 A°) will be injected as a tracer of a different molecular size. Hemoglobin results in similar reaction product as HRP when incubated with DAB. Finally Dextran of varying known

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9. (continued)

sizes--40 A°, 75 A°, 120 A°, can be used as an electron dense probe for capillary permeability (see Progress Report). Hemoglobin and Dextran will be used to validate the use of Peroxidase.

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10. *Space and facilities available (when elsewhere than item 2 indicates, state location):*

Electron Microscopy - Histochemical - Biochemical laboratory, Drew Postgraduate Medical School under direction of principal investigator.

20' x 30'	General Lab	8'x 12'	Microtome
8' x 12'	E.M. Room	6'x 12'	Animal Exposure Room
8' x 12'	Dark Room		

Equipped with:

Hitachi HS 8 Electron Microscope
Durst S-45 EM enlarger
Porter-Blum MT-2 Microtome with Stereomicroscope
3 Diamond knives
General lab centrifuge
Thelco Oven
Vacuum Dessicator (Ladd)
A-O Spencer Cycloptic Stereomicroscope
Leitz Orthoplan-Orthomat photomicroscope
Fully equipped Dark Room
2 Environmental chambers (small animal)
Brinkman TLC Sandwich Chamber
Sorvall Tissue Chopper
New Brunswick Environmental Water Bath Shaker
Kinney Vacuum Evaporator
Mettler Balance
Corning pH Meter

11. Additional facilities required:

LKB E.M. cryokit to cut frozen sections and eliminate loss of lipids without dehydration
Consumable supplies

12. Biographical sketches of investigator(s) and other professional personnel (append):

Date of Birth: R Place of Birth: Philadelphia, Pennsylvania;
Education: University of Pennsylvania A.B. R , M.D. R

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

See (12. append)

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12. (Continued)

Honors: Phi Beta Kappa 1949, Borden Award Undergraduate Medical Research

Intern, Hospital of the University of Pennsylvania	1953-1954
Instructor, Department Pharmacology (Drs. Carl Schmidt & Domingo Aviado) University of Pennsylvania	1954-1955
Resident in Medicine, University of Chicago	1955-1957
Instructor, Department of Medicine, University of Chicago	1957-1958
Assistant Professor of Medicine, University of Chicago	1958-1964
Visiting Professor of Medicine, University of Missouri	1963
Honorary Research Assistant (NSF Fellowship) Dept. Anatomy Kyushu University (Prof. E. Yamada) Fukuoka, Japan	1964-1965
Honorary Research Assistant, Institute of Pathology (Profs. H. Meesen and H. Schultz) Dusseldorf, Germany	1964
Associate Professor of Medicine, University of Chicago	1964-1968
Professor of Medicine, Temple University	1968-

Member:

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BIBLIOGRAPHY

ALBERT H. NIDEN

1. Factors Regulating Capillary Blood (p32) Volume in the Lungs. D. Aviado, F. Bulle, A. H. Niden and C. F. Schmidt. Am. J. Med. Sci. 223:461, 1952.

2. Nervous Influences on Pulmonary Circulation. D. M. Aviado, Jr., A. H. Niden and C. F. Schmidt. Fed. Proc. 15:5, 1956.

3. Effects of Pulmonary Embolism on the Pulmonary Circulation with Special Reference to Arteriovenous Shunts in the Lung. A. H. Niden and D. Aviado. Circulation Research. 4:67, 1956.

4. Goiter and Myxedema due to Iodide Therapy. B. Burrows, A. H. Niden and W. R. Barclay. Amer. Rev. Tuberc. and Resp. Dis. 79:837, 1959.

5. Effects of Drugs on Pulmonary Ventilation and Circulation as Reflected by Changes in Arterial Oxygen Saturation. A. H. Niden and W. R. Barclay. Fed. Proc. 18:114, 1959.

6. Non-uniform Pulmonary Diffusion. B. Burrows, A. H. Niden, I. Moody and P. V. Harper. Fed. Proc. 18:21, 1959.

7. Non-uniform Pulmonary Diffusion as Demonstrated by the Carbon Monoxide Equilibration Technique: Mathematical Consideration. B. Burrows, A. H. Niden, P. Harper and W. R. Barclay. J. Clin. Invest. 39:795, 1960.

8. Non-uniform Pulmonary Diffusion as Demonstrated by the Carbon Monoxide Equilibration Technique: Experimental Results in Man. B. Burrows, A. H. Niden, C. Mittman, R. Talley and W. R. Barclay. J. Clin. Invest. 39:943, 1960.

9. Goiter and Myxedema Due to Iodide Administration. B. Burrows, A. H. Niden, and W. R. Barclay. Ann. Int. Med. 52:858, 1960.

10. The Effects of Drugs on the Pulmonary Circulation and Ventilation as Reflected by Changes in the Arterial Oxygen Saturation. A. H. Niden, B. Burrows and W. R. Barclay. Circulation Research. 8:509, 1960.

11. Determinants of Pulmonary Diffusion in the Experimental Animal. A.H. Niden, B. Burrows, C. Mittman and W. R. Barclay. Amer. Rev. Resp. Dis. 84:132, 1961.

12. The Effects of Acute Atelectasis on the Pulmonary Circulation. A.H. Niden, B. Burrows and W. R. Barclay. Fed. Proc. 20:105, 1961.

13. Percutaneous Pleural Biopsy with a Curreting Needle: Special Reference to Biopsy without Effusion. A. H. Niden, B. Burrows, J. E. Kasik and W. R. Barclay. Amer. Rev. Resp. Dis. 84:37, 1961.

14. Clinical Usefulness of the Single Breath Pulmonary Diffusing Capacity Test. B. Burrows, J. E. Kasik, A. H. Niden and W. R. Barclay. Am. Rev. Resp. Dis. 84:789. 1961.

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15. Effect of Anemia on CO Diffusion in the Perfused Dog Lung. B. Burrows and A. H. Niden. Fed. Proc. 21:443, 1962.

16. Effect on Pulmonary Diffusion of Retrograde and Arterial Blood Perfusions. A. H. Niden and B. Burrows. Fed. Proc. 21:443, 1962.

17. Pulmonary Diffusion in the Dog Lung. A. H. Niden, C. Mittman and B. Burrows. J. Appl. Physiol. 17:885, 1962.

18. Evidence for Pulmonary Venous Obstruction in Pulmonary Emphysema. M. Rabinowitz, A. H. Niden, S. D. Pickard, H. Reissler and F. Navarro Lopez. J. Lab. and Clin. Med. 60:1006, 1962.

19. Effects of Anemia and Hemorrhagic Shock on Pulmonary Diffusion in the Dog Lung. B. Burrows and A. H. Niden. J. Appl. Physiol. 18:123, 1963.

20. Effect of Vascular Pressure and Blood Flow on CO Diffusion in the Perfused Dog Lung. B. Burrows and A. H. Niden. J. Appl. Physiol. 18:722, 1963.

21. The Acute Effects of Atelectasis on the Pulmonary Circulation. A. H. Niden. J. Clin. Invest. 43:810, 1964.

22. American Emphysema and British Bronchitis: A Standardized Comparative Study. C. M. Fletcher, N. L. Jones, B. Burrows and A. H. Niden. Am Rev. Resp. Dis. 90:1, 1964.

23. Clinical Types of Chronic Obstructive Lung Disease in London and Chicago: A Study of 100 Patients. B. Burrows, A. H. Niden, C. M. Fletcher and N. L. Jones. Am. Rev. Resp. Dis. 90:14, 1964.

24. Pulmonary Hypertension in Chronic Obstructive Lung Disease. A. H. Niden, R. A. Duchelle, B. Burrows and M. Rabinowitz. Amer. Rev. Resp. Dis. 90:318, 1964.

25. Chronic Obstructive Lung Disease. I. Clinical and Physiologic Findings in 175 Patients and their Relationship to Age and Sex. B. Burrows, A. H. Niden, W. R. Barclay and J. E. Kasik. Am. Rev. Resp. Dis. 91:521, 1965.

26. Chronic Obstructive Lung Disease. II. Relationship of Clinical and Physiologic Findings to the Severity of Airway Obstruction. B. Burrows, A. H. Niden, W. R. Barclay and J. E. Kasik. Am. Rev. Resp. Dis. 91:665, 1965.

27. Chronic Obstructive Lung Disease. III. Interrelationships of Pulmonary Function Data. B. Burrows, R. H. Strauss and A. H. Niden. Am. Rev. Resp. Dis. 91:861, 1965.

28. The Ultrastructural Effects of Carbon Monoxide Inhalation of the Rat Lung. A. H. Niden and H. Schulz. Virchows Arch. Path. Anat. 339:283, 1965.

29. Chronic Obstructive Lung Disease. A Statement of the Committee on Therapy. R. F. Corpe, S. Grzybowski, F. M. MacDonald, M. M. Newman, A. H. Niden, A. B. Organick and W. Lester (drafted by AHN) Am. Rev. Resp. Dis. 92:513, 1965.

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30. The Effect of Carbon Monoxide Inhalation on the Fine Structure of the Lung. A. H. Niden. Am. Rev. Resp. Dis. 94:490, 1966.

31. The Fine Structure of Bronchiolar Non-Ciliated Cells. A. H. Niden. Fed. Proc. 25:603, 1966.

32. Some Observations on the Fine Structure and Function of the Non-ciliated Bronchiolar Cells. A. H. Niden and E. Yamada. VI International Congress for Electron Microscopy. Vol. II:599, 1966.

33. The Non-ciliated Bronchiolar Cell--Source of Pulmonary Surfactant? A. H. Niden. J. Lab. Clin. Med. 68:1001, 1966.

34. The Non-ciliated Bronchiolar Cell (Clara Cell) as a Source of Pulmonary Surfactant. A. H. Niden and W. Adams. Fed. Proc. 26:497, 1967.

35. The Role of the Bronchiolar and Large Alveolar Cell in Pulmonary Phospholipid Metabolism. A. H. Niden. Science 158:1323, 1967.

36. Effects of Ammonia Inhalation on the Terminal Airways. A. H. Niden. 11th Aspen Conference, pp. 41-44, 1968.

37. Effects of Low Levels Carbon Monoxide on the Fine Structure of the Terminal Airways. A. H. Niden. Am. Rev. Resp. Dis. 103:898, 1971.

38. Observations on Monocytes, Pulmonary Macrophages, and Large Alveolar Cells. A. H. Niden, J. Clin. Invest. 50:70 a, 1971.

39. The Granular Pneumocyte and Pulmonary Fibrosis--Are They Related? (Editorial) A. H. Niden, Chest (in press) 1972.

40. Patterns of Cardiovascular Dysfunction in Chronic Obstructive Lung Disease. B. Burrows, L. J. Kettel, A. H. Niden, M. Rabinowitz, and C. F. Diener. NEJM 286:912, 1972.

41. Role of Pulmonary Surfactant in Disease. A. H. Niden. Pulmonary Care in the Seventies. Hahnemann Symposium. In press. 1972.

42. Pulmonary Surfactant. A. H. Niden. Arch. Env. Health. In press. 1972

43. Effects of Oxygen on Pulmonary Capillary Permeability. Abstract. A. H. Niden, I. Evangelista, R. Belasco. Am. Rev. Res. Dis. 105:1009, 1972.

44. The Lung as a Reservoir for Intravascular Monocytes. A. H. Niden. Manuscript in Preparation.

45. The Chronic Effects of Low Level Carbon Monoxide and Mild Hypoxemia on the Terminal Airways. A. H. Niden. Manuscript in Preparation.

46. The Effects of 100% Oxygen on Pulmonary Capillary Permeability. A. H. Niden. Manuscript in Preparation.

47. Lamellar Body of Large Alveolar Cell.....Artefact of Fixation? A. H. Niden In Preparation

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14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Albert H. Niden, M.D. Principal Investigator	40%	---
Technical		
Graham, Clarence (Includes 17% Fringe Benefits)	100%	R
Sub-Total for A		R

B. Consumable supplies (by major categories)	
Fixatives, Embedding medium, Chemicals, Radioactive material	$1,000
Photographic supplies	1,000
Small animals and care	500
Consumable supplies for E.M.	500
Glassware, plasticware	451
Sub-Total for B	$3,451

C. Other expenses (itemize)	
Travel to scientific meetings	300
50% Service contract for E.M. maintenance	900
Sub-Total for C	$1,200
Running Total of A + B + C	$18,972

D. Permanent equipment (itemize)	
Diamond knife	500
LKB E.M. Cryokit	3,300
Sub-Total for D	$3,800
E. Indirect costs (15% of A+B+C)	2,846
Total request	$25,618

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	3,846	1,200	1,000	3,077	24,590
Year 3	---	4,451	1,200	1,000	3,312	26,391

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
The Ultrastructural Development of Human and Mouse Fetal Lung with Special Reference to Large Alveolar Cell	USPHS	$36,755	July 1, 1973 to June 30, 1975

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to
Charles R. Drew Postgraduate Medical School

Mailing address for checks
1621 E. 120th Street
Los Angeles, Calif. 90059

Principal investigator
Typed Name Albert H. Niden, M.D.
Signature Albert H Niden Date 4/13/73
Telephone R
Area Code Number Extension

Responsible officer of institution
Typed Name Mitchell W Spellman M.D.
Title Dean
Signature Mitchell W. Spellman Date 5/18/73
Telephone (213) 564-5911, Ext. 211
Area Code Number Extension

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